Postembryonic Growth of Two Peripheral Sensory Systems in the Medicinal Leech *Hirudo medicinalis*

LISA GASCOIGNE AND ALISTAIR McVEAN

Department of Biology, Royal Holloway and Bedford New College, University of London, Egham, Surrey TW20 OEX, UK

Abstract. The midbody segments of *Hirudo medicinalis* have two types of sensory cilia that project from the skin into the surrounding water. These are the proposed mechanosensory S cilia and the putative chemosensory G cilia. The cells bearing these cilia are clustered together into structures known as sensilla. The skin of each midbody segment is externally divided into five annuli. Fourteen large sensilla carrying S and G cilia are found on the central annulus of each segment. Small sensilla carrying only G cilia are found on all five annuli. In this paper we show a positive correlation between the size of a neural sensillum and the number of S and G cilia it contains. Any one leech contains a range of sizes of neural sensilla, but the average area increases with the weight of the leech. In contrast, the annular sensilla show only a modest increase in size with leech weight.

Introduction

In both vertebrates (Glucksmann, 1951) and invertebrates (Truman, 1984), during development the final number of cells in the adult nervous system is reduced by cell death. In the ventral horn of the lumbar spinal cord of the frog, as many as 75% of the cells die within 64 days after the hind limbs have emerged (Hughes, 1961). Death of motorneurons in the sphinx moth reduces the number of cells in the adult abdominal ganglia (Truman and Schwartz, 1980). The leech central nervous system is no exception to this process. In *Haemopis marmorata*, the mean number of cells in each ganglion is reduced, over the first 20 days of development, by selective cell death (Stewart and Macagno, 1984).

Some peripheral sensory systems mature through a similar process of cell deletion. Every mammalian species studied loses 50–90% of its retinal ganglion cells (Finlay

and Pallas, 1989). Cell numbers can, however, also increase during postembryonic development. In fish and amphibia, the eyes and the brain grow continuously throughout the life of the animal. New retinal ganglion cells are supplied with synaptic targets by simultaneous growth of the tectum. In insects, new peripheral sensory structures can be added without new cells being added centrally. All insects studied add new chemoreceptors at each additional molt (Chapman, 1982), and new sensory hairs are added to the cerci of crickets at each instar (Murphey and Chiba, 1990). In the medicinal leech, it is possible to compare the postembryonic development of three sensory systems that transduce stimuli at the skin. These are the contact mechanoreceptors whose cell bodies are located within the ganglia (Nicholls and Baylor, 1968); the chemoreceptors on the lips, which consist of multiciliated cells (Elliott, 1987); and the singly ciliated mechanoreceptors that respond to water motion (Friesen, 1981; Young *et al.*, 1981). Both sets of ciliated receptor cells have cell bodies located within the skin; their cilia are known as G cilia and S cilia, respectively (Philips and Friesen, 1982). S cilia range in length from 3 to 9 μm; G cilia from 1 to 2 μm. Sensillum 1 has only short S cilia, whereas other sensilla have long whip-like cilia (DeRosa and Friesen, 1981). So far there is no evidence that the multiciliated afferents in the midbody segments are chemoreceptors.

The cell bodies of the ciliated mechanoreceptors are located, in each segment, on 14 discrete neural sensilla (Fig. 1) that appear, under the dissecting microscope, as pale grey patches. Water flow distorts the cilia (Fig. 2a), setting up action potentials that are transmitted to the central nervous system without an intervening synapse (Philips and Friesen, 1982; Gascoigne and McVean, 1991). Each body segment is externally divided into five annuli. The neural sensilla lie on the central, or neural, annulus. The neural sensilla also carry some chemosen-
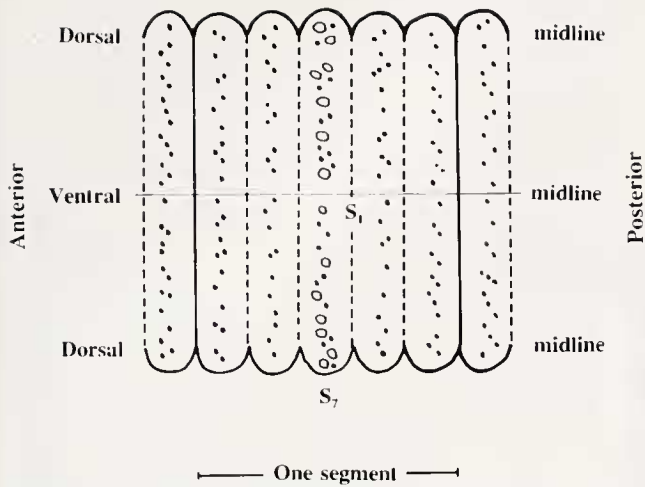
Figure 1. Distribution of neural and annular sensilla on the skin of a typical midbody segment. The skin has been cut along the dorsal midline and pinned flat. There are 14 neural sensilla (clear ellipsoids) on each neural annulus and a variable number of annular sensilla (filled ellipsoids) distributed over all five annuli. The most ventral (S_1) and most dorsal (S_7) sensilla are labeled.

sory G cilia (DeRosa and Friesen, 1981), but small (annular) sensilla (Fig. 2b) with only G cilia are also found on both the central annulus and the other four annuli of each segment (McVean et al., 1990). Unlike the neural sensilla, the annular sensilla are variable in number and position; they are also more numerous (McVean et al., 1990).

Embryogenesis in *Hirudo medicinalis* is complete after 30 days. Neurogenesis and cell death in the central nervous system are complete after 12 days, after which the number of mechanosensory cells in the segmental ganglia becomes fixed (DeReimer and Macagno, 1989). Yet leeches continue to grow postembryonically; the largest leeches we used in this study were about 230 times heavier than the smallest leeches. These facts raise the following questions. Do S and G cilia-bearing cells maintain their initial density in the skin by postembryonic recruitment of additional sensory cells as the leech grows, or are the numbers of ciliated cells, like those of the centrally located neurons, fixed at 12 days? If, on the other hand, the numbers of ciliated cells in the sensilla change as the leech grows, do the annular and neural sensilla change in the same way?

To answer these questions, we examined the change in diameter of the neural and annular sensilla in juvenile, postjuvenile, and mature adult leeches, having first established that there is a positive correlation between the area of a neural sensillum and the number of cilia in it.

Materials and Methods

Animals

Adult *Hirudo medicinalis*, approximately 6 months old, and unfed juveniles, 3 weeks old, were obtained from

Biopharm (UK) Ltd. and maintained in filtered pond water at 12°C.

SEM preparation

We started with a single batch of 20 three-week-old leeches. Two leeches from this batch were anesthetized by immersion in leech Ringer (Muller et al., 1981) con-
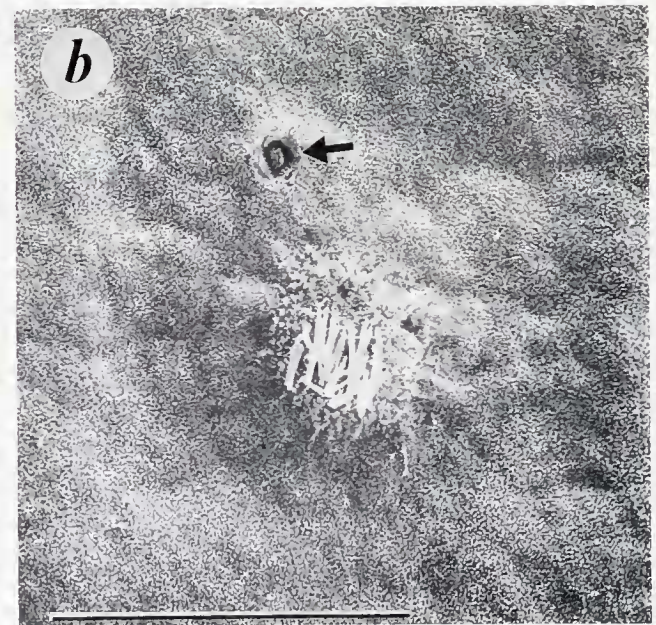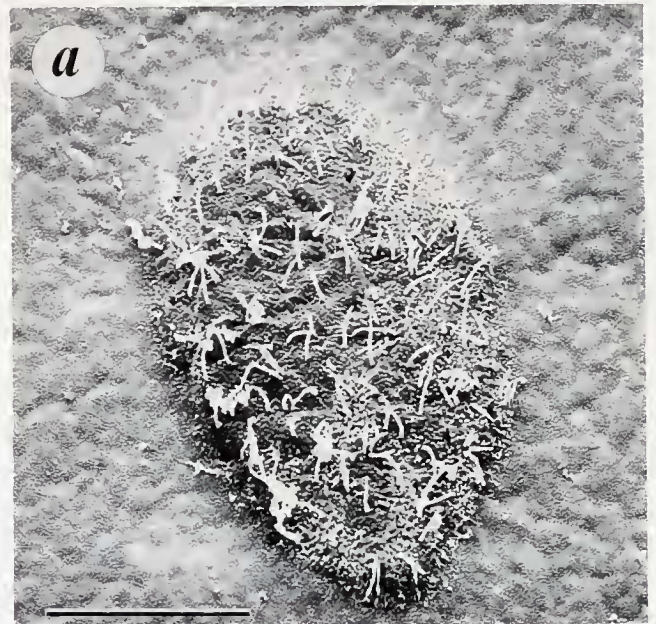
Figure 2. Scanning electron micrograph of a neural (a) and an annular (b) sensillum. Both S and G cilia are present on the neural sensilla, but the annular sensilla carry only G cilia. In the living animal, both neural and annular sensilla are raised above the surface of the skin on circular mounds. The arrow shows a pore, which are often associated with annular sensilla. Scale bars 20 μm.
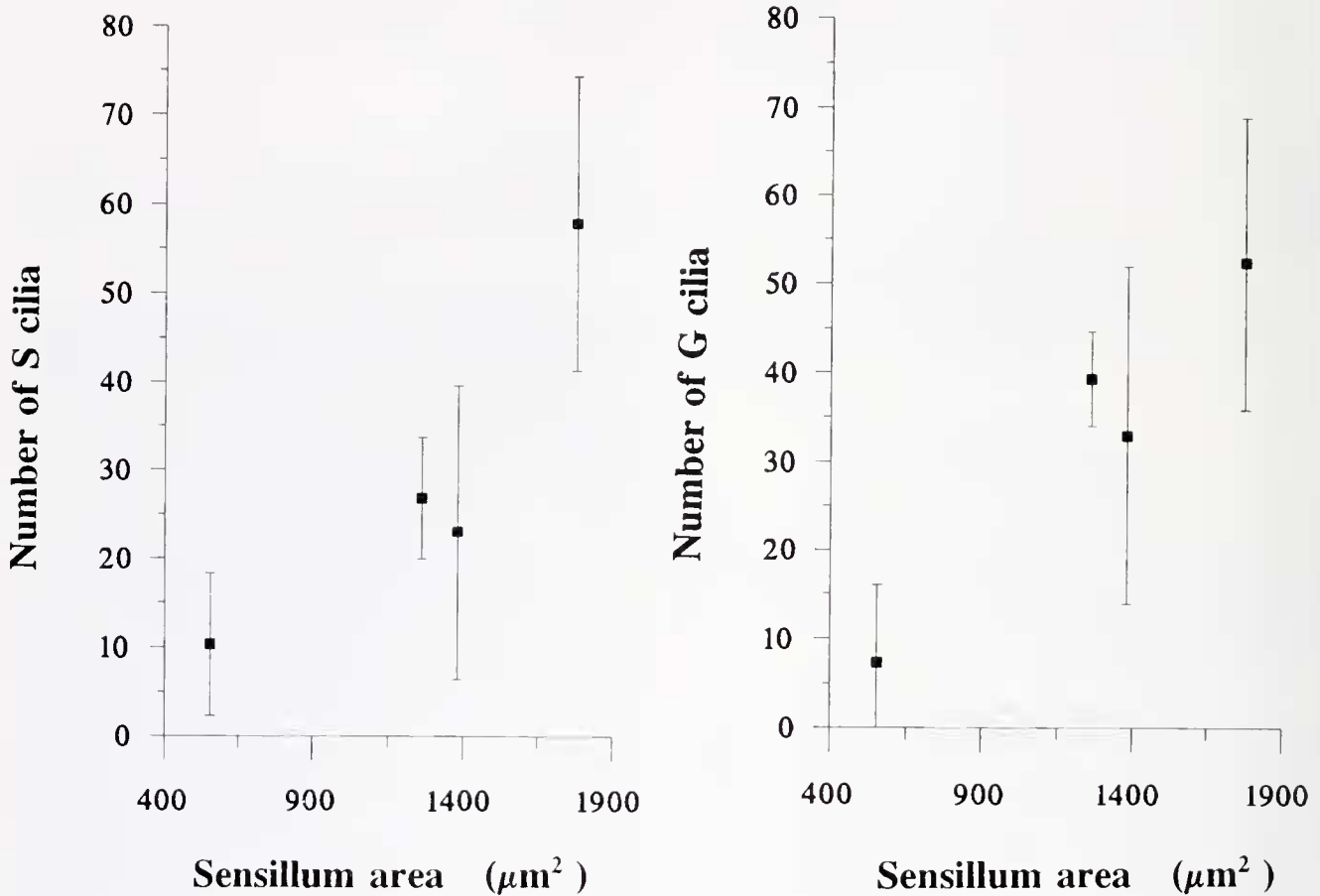
Figure 3. The number of S cilia (left) or G cilia (right) in a sensillum is directly correlated with sensillum size. Data are from four leeches whose weights ranged from 94 to 2600 mg. Each point represents the mean (±1 SD) for 10 neural sensilla from each leech. The sensilla were selected at random.

taining 8% ethanol and then weighed after their body surface was dried. The leeches were spread out, dorsal side uppermost, and pinned onto sheets of dental wax. We used a large number of pins around the perimeter in an effort to stretch the body wall evenly. Mucus was removed by rinsing the surface of the skin in 8% ethanol. Preparations were fixed in 3% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 7.2, for 2 h, dehydrated through a graded series of ethanol, and critical-point dried. Preparations were mounted on stubs, splutter coated with gold palladium, and viewed in a Cambridge S-100 scanning electron microscope. We inspected adjacent midbody segments in the region of the genital openings and measured the diameter of sensilla visible on their ventral surface. When searching for sensilla, we viewed the surface of the skin at a magnification of 750× and checked the identity of possible sensilla at higher magnification. Searching started midventrally; the stub was moved one frame at a time, working from the posterior edge of the annulus to the anterior edge, at which point the stub was moved one frame away from the midline and the search continued in the opposite direction.

This procedure was repeated with two more leeches from the original batch at weekly intervals for a further 7 weeks (10th postembryonic week). The remaining leeches were fed, to satiation, in their 11th postembryonic (PE) week. Two of these were examined in their 12th PE week, and the last two leeches were fed a second time in the 13th PE week and their sensilla measured in the 15th PE week.

A further six leeches were prepared for scanning electron microscopy. Two were large adults (4148 and 5454 mg) used to confirm that neural sensilla continue to grow beyond the 15th PE week. The other four leeches (94 and 1430 mg) were used to define the relationship between the area of a sensillum and the number of externally ciliated cells it contains. Adult leeches were prepared by first removing their body contents and then dividing the skin into anterior and posterior halves. Fixation and preparation was then as for juvenile leeches.

Results

Tissue shrinkage in leech skin due to fixation has been estimated to be 16–29% (DeRosa and Friesen, 1981; El-
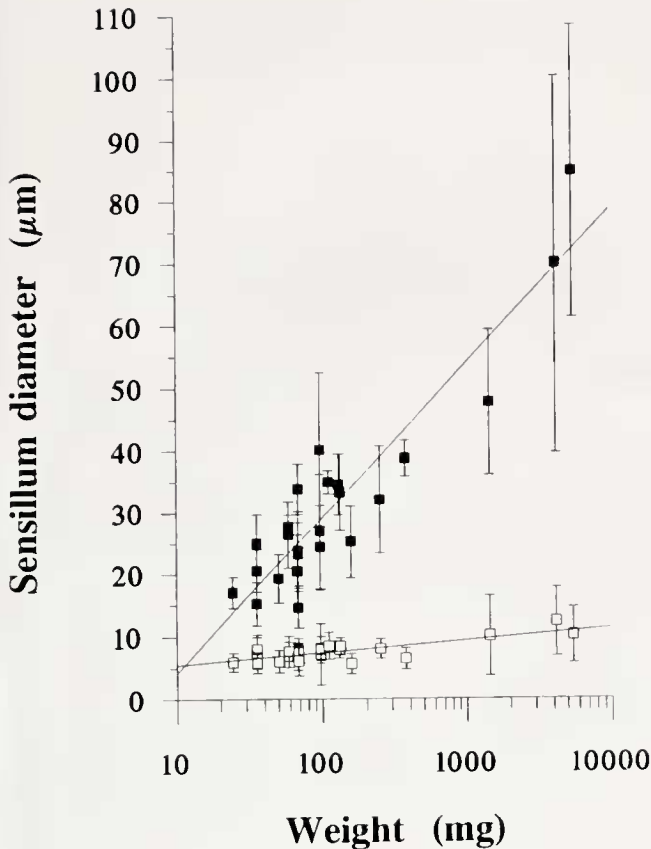
Figure 4. Mean (±1 SD) of neural (■) and annular (□) sensilla on midbody segments of leeches of different weights plotted against log weight of the leech. The size of both neural and annular sensilla is directly correlated with the size of the leech, but the size increase of the annular sensilla over this weight range is small compared with the size increase of the neural sensilla over the same weight range. A line of best fit is drawn through each set of data points.

liott, 1987). Measurements of sensilla dimensions are therefore relative rather than absolute.

Relationship between sensillum area and number of cilia

We measured the major and minor diameter of 10 annular sensilla and 10–31 neural sensilla in each of four leeches whose weights ranged from 94 to 2600 mg. We counted the number of S and G cilia in the neural sensilla and the number of G cilia in the annular sensilla (G cilia from one cell become clumped together during preparation for scanning electron microscopy, and each clump was counted as one). For the neural sensilla, area was positively correlated with number of both S ($r^2 = 0.79$) and G ($r^2 = 0.95$) cilia (Fig. 3). Annular sensilla varied little in diameter between leeches (Fig. 4). DeRosa and Friesen (1981) found similar numbers of S cilia per sensillum, but our numbers for G cilia are higher than theirs.

Sensillum size in postembryonic to adult leeches

After demonstrating a positive correlation between the area of a neural sensillum and the number of S and G cilia it contains, we used the diameter of both neural and annular sensilla as an indirect measure of the number of S and G ciliated cells in leeches whose weights spanned a range from immediately postembryonic to large adult. We measured the major diameter of 176 neural sensilla and 712 annular sensilla in 23 leeches weighing from 24 to 5450 mg. Over this weight range, leech weight is positively correlated with the diameter of both neural ($r^2 = 0.89$) and annular ($r^2 = 0.78$) sensilla (Fig. 4). Though significant, the growth of annular sensilla is small. The ratio between the average area of the annular sensilla in the smallest and largest leech was 1:4, and the similar ratio for the neural sensilla was 1:24.

The area of the neural sensilla in any one leech varies and is positively correlated with the number of ciliated cells it contains (Fig. 5).
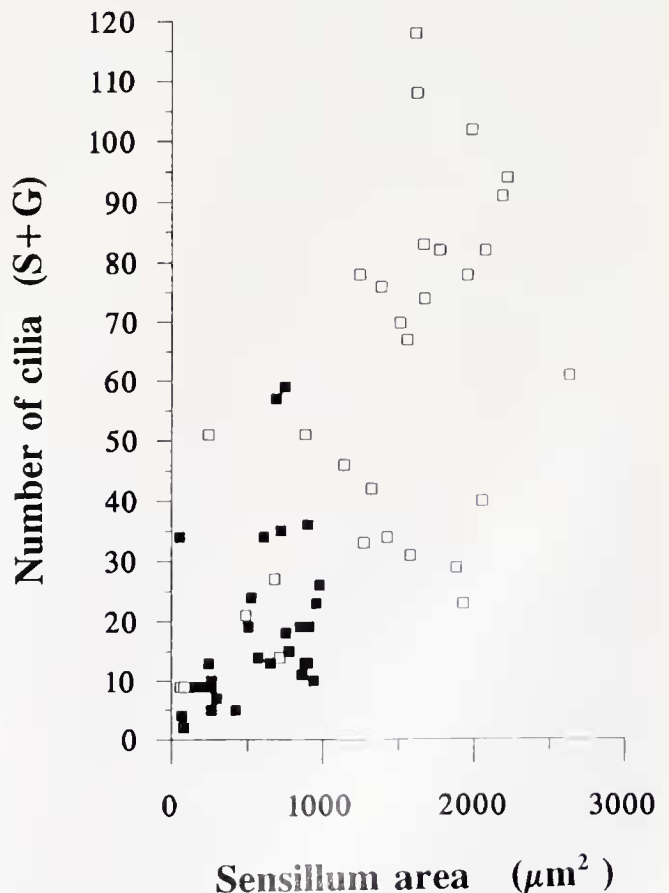

Figure 5. The size of the neural sensilla in any one leech is variable. Here the numbers of S and G cilia are plotted against sensillum area for two leeches of different weights. ■ = 500 mg leech. □ = 2600 mg leech. The sizes of the neural sensilla in these two leeches overlap.

Discussion

We have shown positive correlations between the area of a neural sensillum and the number of S and G cilia that it contains and between the weight of a leech and the diameter of its neural sensilla. Over the range that we considered, the weight of the leech increased about 230 times, the area of the neural sensilla increased about 24 times, and the area of the annular sensilla increased about 4 times. We conclude that, as leeches grow, the neural sensilla increase in area as more S and G cilia are added.

New G-cilia-bearing cells clearly are recruited into the neural sensilla, so their number is not fixed by the end of embryogenesis. The modest growth in the area of the annular sensilla may be compensated for by the addition of new annular sensilla. Because the skin was invariably coated with patches of mucus, we were unable to assess the number of annular sensilla for any one leech.

The increase in the number of chemoreceptors and mechanoreceptors in insects with successive instars is well documented (Murphey and Chiba, 1990; Chapman, 1982). The total number of olfactory pegs and contact chemoreceptor pegs in the cockroaches *Leucophaea maderae* and *Periplaneta americana* increases dramatically at each instar, but the density of both types of sensory receptors remains constant. Only in the adult male does the density double (Chapman, 1982).

During insect development there is a dynamic relationship between the number of peripheral sensory cells and the structure of the dendritic arbor of the interneuron onto which the sensory cells synapse. When a cercus was removed from embryonic grasshoppers that had completed 60% of their development, the medial giant interneuron developed 30% fewer dendritic branches (Shankland *et al.*, 1982).

Our data suggest that new peripheral sensory cells continue to be added throughout the life of the leech. Because the S-cilia-bearing cells probably synapse directly onto T cells as well as onto first order interneurons (Gascoigne and McVean, 1991), the increase in size of the T cell arbor in larger leeches (DeReimer and Macagno, 1989) may partly reflect increased demands for synaptic space by the peripheral sense organs.

Other authors (Peinado, 1988; Peinado *et al.*, 1990) have shown that new axons are added to peripheral nerves after embryogenesis is complete, surmising that these additions were from sensory structures. We have confirmed that new sensory structures are added to the skin of the leech after embryogenesis and, because these must communicate with the central nervous system via axons, they must contribute to the new axon complement observed by Peinado *et al.* (1990). We have also shown that new sensory structures are added throughout adult life. Although the additions include both S and G ciliated sensory

structures, the pattern of development is different for the cells within annular and neural sensilla.

Literature Cited

Chapman, R. F. 1982. Chemoreception: the significance of receptor numbers. Pp. 247–356 in *Advances in Insect Physiology*, Vol. 16, M. J. Berridge, J. E. Treherne, and V. B. Wigglesworth, eds. Academic Press.

DeReimer, S. A., and E. R. Macagno. 1989. Quantitative studies on the growth of neuronal arbors. Pp. 11–31 in *Perspectives in Neural Systems and Behaviour*, T. J. Carew and D. B. Kelly, eds. Alan R. Liss.

DeRosa, S. Y., and W. O. Friesen. 1981. Morphology of leech sensilla: observations with a scanning electron microscope. *Biol. Bull.* 160: 383–393.

Elliott, E. J. 1987. Morphology of chemosensory organs required for feeding in the medicinal leech. *J. Morph.* 192: 181–187.

Finlay, B. L., and S. L. Pallas. 1989. Control of cell number in the developing mammalian visual system. *Prog. Neurobiol.* 32: 207–234.

Friesen, W. O. 1981. Physiology of water motion detection in the medicinal leech. *J. Comp. Physiol.* 92: 255–275.

Gascoigne, L., and A. R. McVean. 1991. Water movement sensitive cells in leech C.N.S. *Phil. Trans. R. Soc. Lond. B* 332: 261–270.

Glucksmann, A. 1951. Cell deaths in normal vertebrate ontogeny. *Biol. Rev.* 26: 59–86.

Hughes, A. F. 1961. Cell degeneration in the larval ventral horn of *Xenopus laevis* (Daudin). *J. Embryol. Exp. Morphol.* 9: 269–284.

McVean, A. R., L. Gascoigne, and A. Page. 1990. The external structure and distribution of sensilla in the medicinal leech. *Acta Zool.* 71: 161–167.

Muller, K. J., J. G. Nicholls, and G. S. Stent. 1981. *Neurobiology of the Leech* Cold Spring Harbour Laboratory.

Murphey, R. K., and A. Chiba. 1990. Assembly of the cricket cercal sensory system: genetic and epigenetic control. *J. Neurobiol.* 21: 120–137.

Nicholls, J. G., and D. A. Baylor. 1968. Specific modalities and receptive fields of sensory neurons in C.N.S. of the leech. *J. Neurophysiol.* 31: 740–756.

Peinado, A. 1988. Axon guidance and fasiculation in the peripheral nervous system of the leech *Hirudo medicinalis.* Ph.D. Thesis, Columbia University.

Peinado, A., B. Zipser, and E. R. Macagno. 1990. Segregation of afferent projections in the central nervous system of the leech *Hirudo medicinalis. J. Comp. Neurol.* 301: 232–242.

Philips, C. E., and W. O. Friesen. 1982. Ultrastructure of the water-movement-sensitive sensilla in the medicinal leech (*Hirudo medicinalis*). *J. Neurobiol.* 13: 473–486.

Stewart, R. R., and E. Macagno. 1984. The development of segmental differences in cell number in the CNS of the leech. *Soc. Neurosci. Abstr.* 10: 512.

Shankland, M., D. Bentley, and C. S. Goodman. 1982. Afferent innervation shapes the dendritic branching pattern of the medial giant interneuron in grasshopper embryos raised in culture. *Dev. Biol.* 92: 507–520.

Truman, J. W. 1984. Cell death in invertebrate nervous systems. *Ann. Rev. Neurosci.* 7: 171–188.

Truman, J. W., and L. M. Schwartz. 1980. Peptide hormone regulation of programmed death of neurons and muscle in an insect. In *Peptides. Integrators of Cell and Tissue Functions. Soc. Gen. Physiol. Series*, Vol. 25, F. E. Bloom, ed. Raven Press, New York.

Young, S. R., R. D. Dedwylder, and W. O. Friesen. 1981. Response of the medicinal leech to water waves. *J. Comp. Physiol. A* 144: 111–116.